Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information for

High resolution Atomic Force Microscopy of double-stranded RNA

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SUPPLEMENTARY METHODS.

Vertical noise measurement.

To determine the instrumental noise level in the vertical direction (z), we approached the tip to a freshly-cleaved mica surface under AM-AFM experimental conditions for high resolution imaging (see Experimental section in the main text). Once in range, we then recorded the height channel for two minutes at a data acquisition bandwidth of 4.5 Hz. During the measurement the scan size was set to zero. Height values were represented as a histogram and fitted to a Gaussian function with a standard deviation value (RMS as the mean value is 0) of 0.3 Å.

Measuring and imaging parameters.

The relevant measuring and imaging parameters for the three imaging modes employed in this study are included in **Table S1**. Note that DAM-AFM requires a larger number of imaging parameters because this method has three basic variables (oscillation amplitude, phase, and driving amplitude) in contrast with AM-AFM and JM+ that require control of just a single one (oscillation amplitude in AM-AFM, and normal force in JM+). A description of the operational configurations including the feedback diagrams of AM-AFM, DAM-AFM, and JM+, is shown in cartoons in **Figure S3** (adapted from [1])**.** A comprehensive description of the three methods is included in the figure caption.

Substrate roughness periodicity analysis.

We analyzed the periodicity of substrate roughness by performing a Fourier Analysis of images free of DNA/RNA molecules (**Fig. S5**). A typical image of a clean area is shown in **Fig. S5a**. The RMS roughness of this image was 0.33±0.06 nm (**Fig. S5b**). To analyze the corrugation amplitude as a function of the periodicity we computed the angle-integrated power spectral density (PSD) of the image (**Fig. S5c**). These data give information on the contribution to the signal of the different wavelengths (periodicities) of the substrate. The angle-integrated PSD on these clean regions did not show the predominance of any particular wavelength. We observed that below 10 nm (k = 0.1 nm^{-1}) wavelength periodicity the roughness amplitude was already at least one order of magnitude lower than the amplitude observed at the maximum possible wavelength given by the size of the image (~100 nm, k=0.01 nm^{-1}) (**Fig. S5d**). Moreover, at the periodicities of interest of our study, i.e., 3.4 nm and 3.1 nm for dsDNA and dsRNA (~ k = 0.3^{-1}) the amplitude of the corrugation was negligible (**Fig. S5d inset**). Additionally, dsDNA or dsRNA molecules are insensitive to any residual short-length corrugation of the substrate because of the large persistence length of these polymers (around 50 nm for DNA and 62 nm for dsRNA) [2] [3].

SUPPLEMENTARY TABLE.

Table S1 Measuring and imaging parameters employed in this study

AM-AFM	
Scan rate (lines·s^{-1})	3-5
Main feedback channel	Amplitude
Set point main feedback	0.5-0.8 nm
P,I values main feedback*	20,10
DAM-AFM	
Scan rate (lines·s^{-1})	4-8
Main feedback channel	Dissipation
Set point main feedback	0.2-0.5 fW
P,I values main feedback*	50,25
Set point Amplitude (nm)	0.6
P,I values Amplitude*	8000,80
P,I values Phase*	6,12
JM+	
Scan rate (lines·s^{-1})	3-4
Main feedback channel	Force
Set point main feedback	30-40 pN
P,I values main feedback*	40,20
Amplitude of Z excursion (nm)	15-35
Frequency of Z excursion (kHz)	0.5-1
Common parameters	
Image size (nm)	50-150
Number of points per line	512
Pixel resolution (nm·pix^{-1})	0.1-0.3
Cantilevers	Biolever mini BL-AC40TS-C2 k = 0.09 N/m, f_0 (liquids) = 25 kHz, nominal tip radius = 8 nm
Cantilever free amplitude for approach (nm)	~ 7
Approach set point (nm)	~ 5 (75% of free amplitude)
Optical sensitivity (nm/V)	9

*P,I feedback values will vary when using different microscopes. The values shown here can be seen as a reference for Nanotec Electronica Cervantes AFM users.

SUPPLEMENTARY FIGURES.

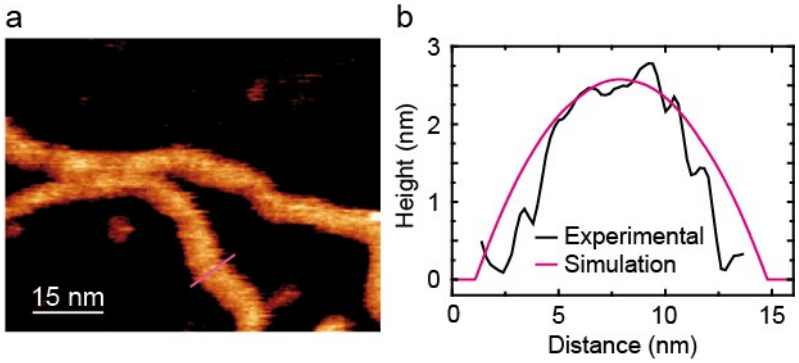


Figure S1. *dsRNA molecules measured using a tip with radius close to the nominal value.* (**a**) AFM topographic image where no helical resolution can be seen along the molecules. Color scale (from dark to bright) is adjusted to enhance the corrugation (2.5 nm total range). (**b**) Comparison of cross-sectional profiles corresponding to line in (a) and an 8 nm radius tip-dilated simulation. It can be observed that the radius of the tip used for the acquisition is close to the nominal 8 nm value and not enough to provide high resolution.

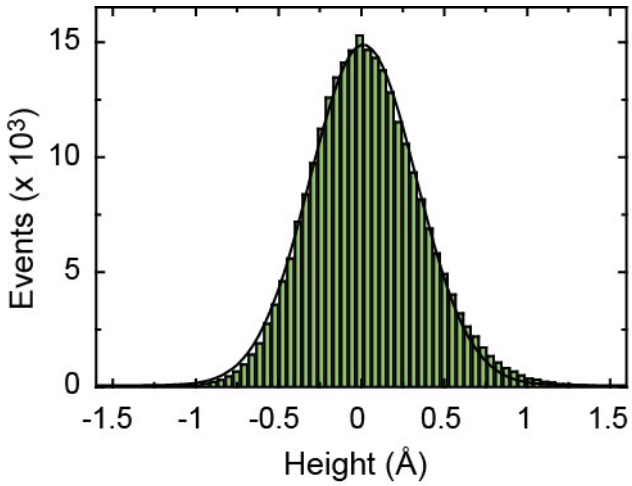
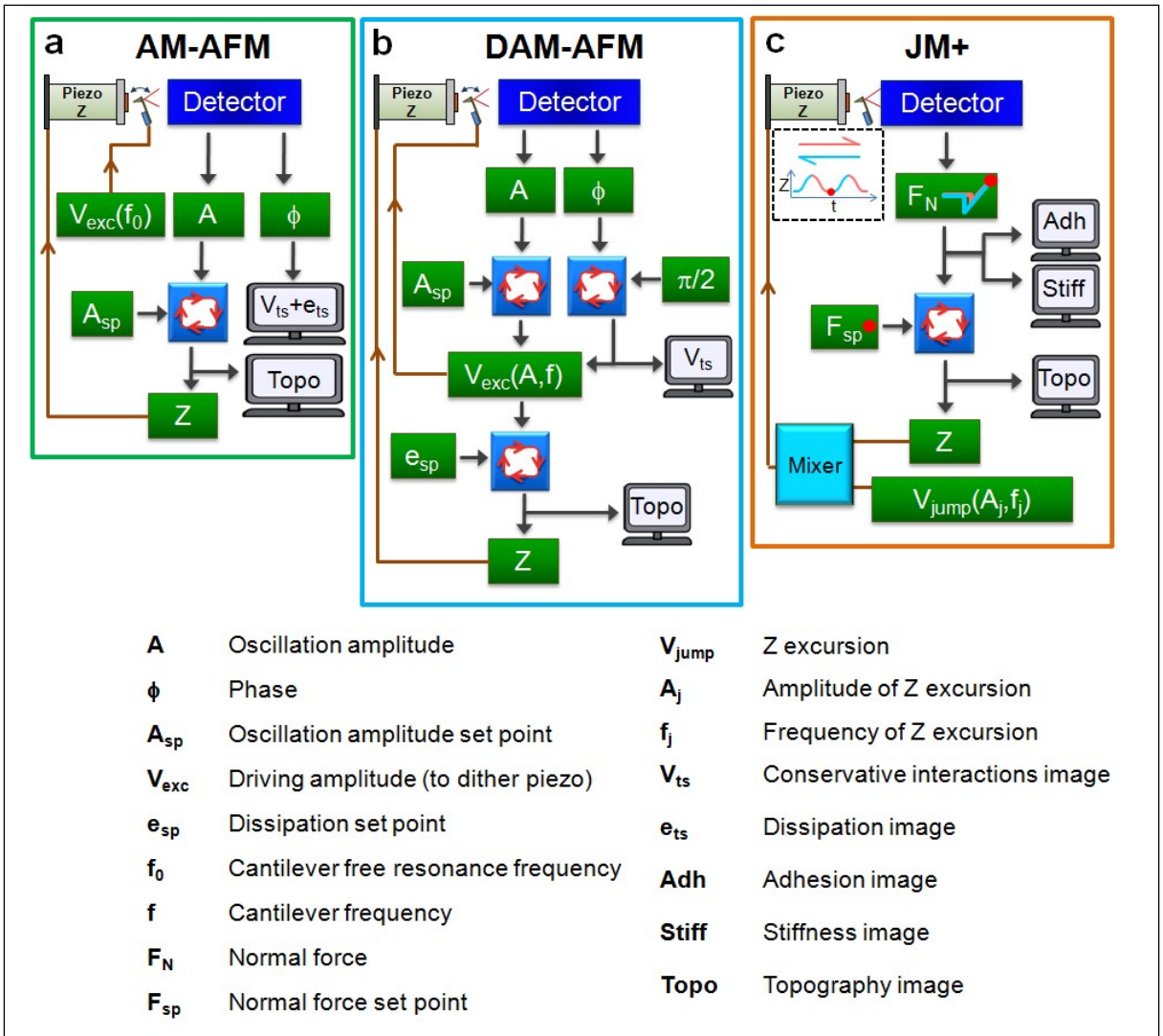


Figure S2. *Noise in AFM height measurement*. Gaussian fit leads to a RMS noise value of 0.32 Å.



A	Oscillation amplitude	$\mathbf{V_{jump}}$	Z excursion
ϕ	Phase	$\mathbf{A_j}$	Amplitude of Z excursion
$\mathbf{A_{sp}}$	Oscillation amplitude set point	$\mathbf{f_j}$	Frequency of Z excursion
$\mathbf{V_{exc}}$	Driving amplitude (to dither piezo)	$\mathbf{V_{ts}}$	Conservative interactions image
$\mathbf{e_{sp}}$	Dissipation set point	$\mathbf{e_{ts}}$	Dissipation image
$\mathbf{f_0}$	Cantilever free resonance frequency	**Adh**	Adhesion image
f	Cantilever frequency	**Stiff**	Stiffness image
$\mathbf{F_N}$	Normal force	**Topo**	Topography image
$\mathbf{F_{sp}}$	Normal force set point		

Figure S3. *Feedback diagrams of the three measuring modes used in this study.* (**a**) AM-AFM. The cantilever is oscillated at its free resonance frequency (f_0) and the amplitude is used as the controlled input for the topography feedback. The phase produces a map of conservative (V_{ts}) + dissipative (e_{ts}) forces. (**b**) DAM-AFM. There are two feedback branches, a short one (feedback on the phase) and a long one (topography feedback). The short branch is a phase-lock loop, which produces a map of the conservative force (V_{ts}) by keeping the system in resonance (phase = $\pi/2$) varying the cantilever oscillation frequency (f) accordingly. The long branch uses the amplitude as the process variable, and the regulated variable is the dissipation, which is used as the controlled input for the topography feedback. (**c**) JM+. The cantilever is not oscillated, the system performs a quick force *vs.* distance curve (FZ) at each point of the scanned area, moving the tip laterally at the farthest tip sample distance minimizing lateral forces. The FZ is performed using a sinusoidal voltage wave (V_{jump}, with amplitude A_j and frequency f_j) that is applied to the scanning piezoelectric. Adhesion and Stiffness maps are produced from the FZ. The Normal force is directly used as the controlled input for the topography feedback.

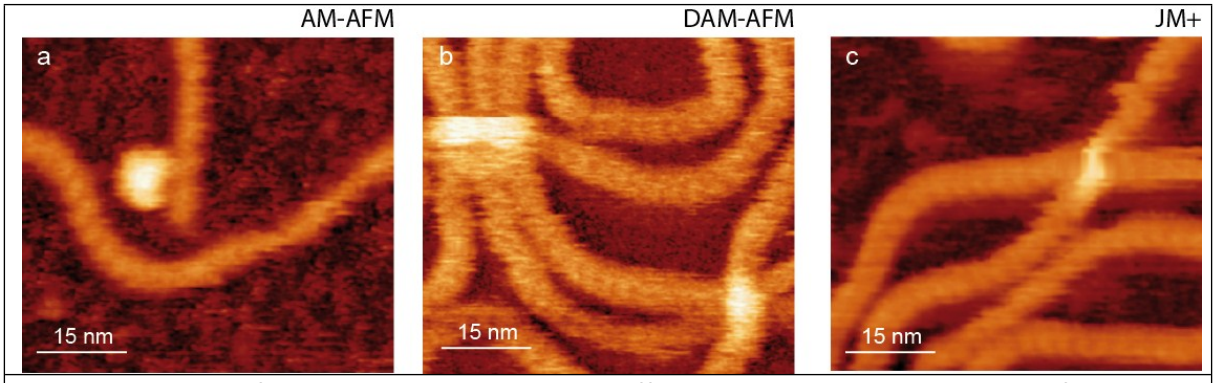


Figure S4. *Images of dsRNA molecules taken with different acquisition modes shown at full height color scale.* Images correspond to those of **Fig.3**. (**a**) AM-AFM. (**b**) DAM-AFM. (**c**) JM+. Color scale (from dark to bright) is 5 nm total range.

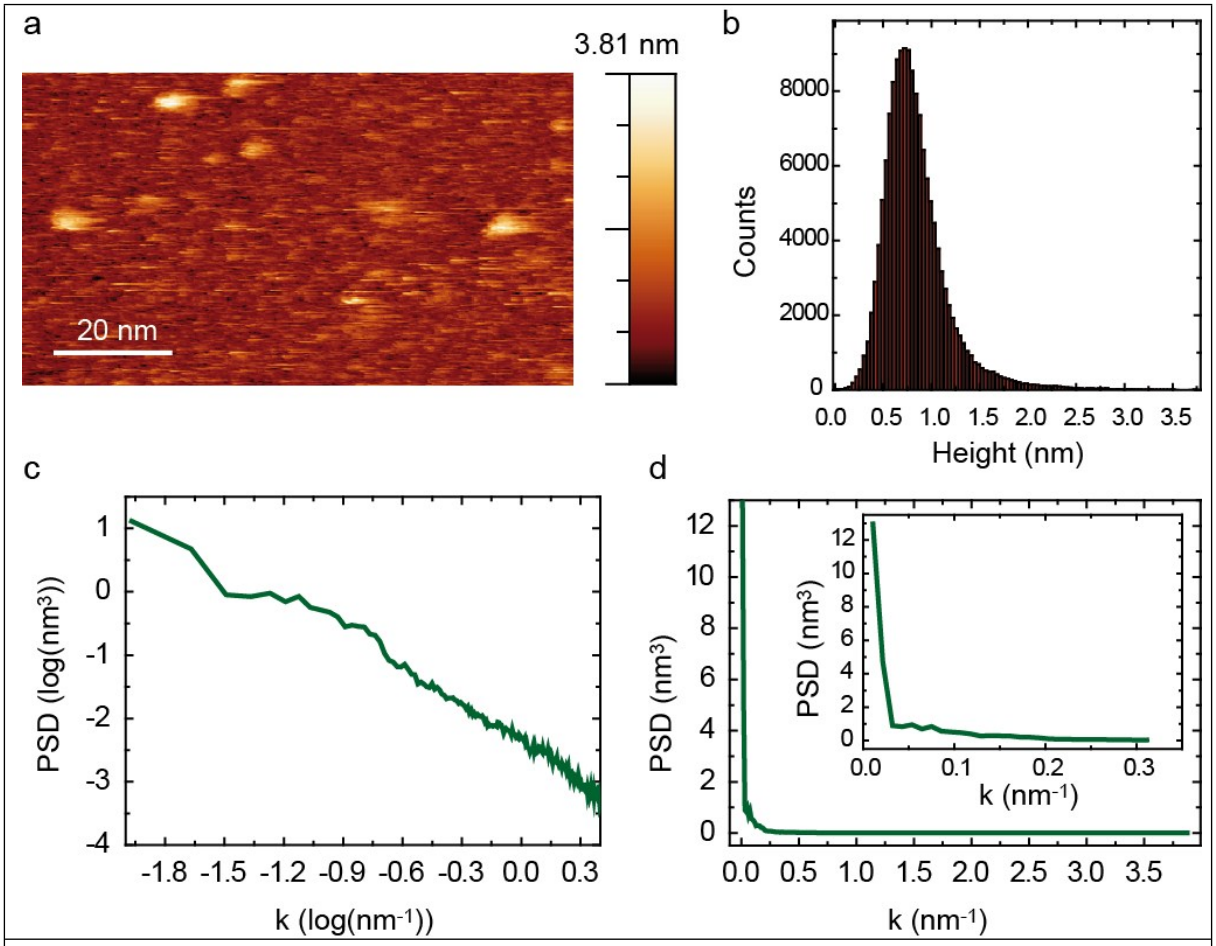


Figure S5. *Substrate roughness periodicity analysis.* (**a**) Image of a molecule-free area. (**b**) Height histogram of the whole image. (**c**) Angle integrated log-log PSD of the image shown in (a). (**d**) Linear plot of the angle-integrated PSD. The inset shows a magnification for low k values.

SUPPLEMENTARY REFERENCES.

1. M. Jaafar, D. Martinez-Martin, M. Cuenca, J. Melcher, A. Raman and J. Gomez-Herrero, *Beilstein journal of nanotechnology*, 2012, **3**, 336-344.
2. S. B. Smith, L. Finzi and C. Bustamante, *Science*, 1992, **258**, 1122-1126.
3. J. A. Abels, F. Moreno-Herrero, T. van der Heijden, C. Dekker and N. H. Dekker, *Biophys J*, 2005, **88**, 2737-2744.